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Characterization of 21 strains of *Bacillus anthracis*

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Abstract

Twenty-one strains of *Bacillus anthracis* currently held in the culture collection at DRES were characterized by colonial morphology, antibiotic sensitivity and Biolog™ metabolic identification profiles. Aside from *Bacillus anthracis* strain Vollum which appeared more "crusty" on blood plates, the other strains were similar, giving a characteristic "Greenland" appearance (rough edge, off-white to tan colouration). Most strains were resistant to ceftazidime, colistin, bacitracin, polymyxin B and sensitive to the other antibiotics tested, such as ciprofloxacin. Biolog™ metabolic identification profiles were prepared for each of the 21 strains of *Bacillus anthracis* as part of the identification database being developed for the "Defence Reference Center for Infectious Agents (DRCIA) (a center of expertise for endemic and exotic diseases of concern to the Canadian Forces) at DRES. A representative strain (Sterne) of *Bacillus anthracis* was used to test the disinfectant sensitivity of both *Bacillus anthracis* vegetative cells and spores. *Bacillus anthracis* vegetative cells were sensitive to 1:100 and 1:10 diluted Savlon™ as well as 1:100 and 1:10 diluted household bleach while the spores were only sensitive to 1:10 diluted household bleach.

Résumé

Vingt et une souches de *Bacillus anthracis* conservées actuellement dans la collection de cultures du CRDS ont été caractérisées par la morphologie des colonies, la sensibilité aux antibiotiques et les profils d'identification métabolique Biolog™. À part la souche Vollum de *Bacillus anthracis* qui avait une apparence plus « croûtée » sur les plaques au sang, les autres souches avaient une apparence « herbeuse » semblable (bord rugueux, couleur blanc neutre à havane). La plupart des souches étaient résistantes à la ceftazidime, à la colistine, à la bacitracine, à la polymyxine B et sensibles aux autres antibiotiques testés, comme la ciprofloxacine. Des profils d'identification métabolique Biolog™ ont été préparés pour chacune des 21 souches de *Bacillus anthracis*, dans le cadre de la base de données d'identification mise au point pour le projet de centre de référence pour la défense contre les agents infectieux (CRDAI) (un centre d'expertise sur les maladies endémiques et exotiques intéressant les Forces canadiennes), au CRDS. Une souche représentative (Sterne) de *Bacillus anthracis* a été utilisée pour tester la sensibilité des cellules végétatives et des spores de *Bacillus anthracis* aux désinfectants. Les cellules végétatives de *Bacillus anthracis* étaient sensibles aux dilutions 1:100 et 1:10 de Savlon™ et à des dilutions 1:100 et 1:10 d'eau de Javel, alors que les spores n'étaient sensibles qu'à la dilution 1:10 d'eau de Javel.

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Executive summary

Bacillus anthracis, the causative agent of anthrax, has long been known as the "classic" Biological Warfare (BW) agent. Its ease of growth, survival characteristics of its spore, its lethality in pneumonic form, and its past weaponization by several nations have all contributed to its reputation. In light of this, it was certainly no surprise to learn of Iraq's admission that it had weaponized this agent for use during the Gulf War Crisis in 1990.

As a disease, anthrax has received a great deal of press coverage since 1990 and has become a popular choice for incidents of "bioterrorism", although most of these cases to date have proven to be hoaxes. In one incident, however, an American in Las Vegas declared that he had a vial of *Bacillus anthracis*, and indeed he did much to the dismay of authorities. It was later learned that the anthrax in question during this incident was the vaccine strain and not a virulent strain as the individual had implied.

With its potential as both a military and terrorist threat, *Bacillus anthracis* is an integral part of the research program at DRES. It is used as a model system in the study of medical countermeasures, identification and decontamination studies. As part of our safety protocols we have undertaken to characterize the antibiotic and disinfectant sensitivities of *Bacillus anthracis*, as well as the other Risk Group 3 bacterial agents at DRES. This ensures that this information will be quickly available in the event of accidental exposure at DRES, and available to the Canadian Forces (CF) and other government agencies in the event of military or terrorist use. We are also in the process of establishing an identification capability for Risk Group 3 agents of interest to the CF as part of the Defence Reference Center for Infectious Agents (DRCIA).

Although vegetative cells of *Bacillus anthracis* were sensitive to diluted Savlon or 1:100 bleach, its spores were very resistant. *Bacillus anthracis* spores, however, were destroyed in less than a minute after coming into contact with 1:10 household bleach. Biolog™ metabolic profiles were determined for all 21 anthrax strains held at DRES. These profiles will serve as a key part of planned DRCIA identification capabilities. Antibiotic sensitivity profiles confirm that all anthrax strains held at DRES are sensitive to a variety of antibiotics, including Ciprofloxacin which is the only antibiotic approved by the FDA in the US for treatment of inhalational anthrax.

Kournikakis, B.; Bateman, C.; Cherwonogrodzky, J.W. 2000. Characterization of 21 strains of *Bacillus anthracis*. TM-2000-157 Defence Research Establishment Suffield.

Sommaire

Bacillus anthracis, l'agent causal du charbon bactéridien, est connu depuis longtemps comme l'agent de guerre biologique « classique ». Sa croissance rapide, l'aptitude de ses spores à survivre, la létalité de la forme pulmonaire et le fait que plusieurs États l'ont déjà utilisé comme arme sont tous des éléments qui ont contribué à sa réputation. À la lumière de ces faits, il n'a pas été surprenant d'apprendre que l'Iraq a admis avoir utilisé cet agent comme arme durant la guerre du Golfe en 1990.

La maladie causée par cet agent, le charbon bactéridien, a été amplement commentée par la presse depuis 1990 et est devenue un choix populaire pour les « bioterroristes », mais la plupart des cas jusqu'à maintenant se sont révélés des canulars. Lors d'un incident, toutefois, un Américain à Las Vegas s'est dit en possession d'une fiole de *Bacillus anthracis*, et on a constaté que c'était vrai, à la grande consternation des autorités. On a appris plus tard que l'agent du charbon bactéridien en question était une souche de vaccin et non la souche virulente comme l'individu l'avait laissé entendre.

Étant donné la menace d'ordre militaire et terroriste que *Bacillus anthracis* laisse planer, cet agent fait partie intégrante du programme de recherche du CRDS. On l'utilise comme système modèle dans l'étude des mesures de prévention médicales, l'identification et les études de décontamination. Dans le cadre de nos protocoles de sécurité, nous avons entrepris de caractériser la sensibilité de *Bacillus anthracis* aux antibiotiques et aux désinfectants, ainsi que celle des autres agents bactériens du Groupe de risque 3 conservés au CRDS. Ainsi, ces données seront facilement accessibles dans l'éventualité d'une exposition accidentelle au CRDS, et les Forces canadiennes (FC) et les autres organismes gouvernementaux pourront également les consulter en cas d'utilisation militaire ou terroriste. Nous sommes également en train d'établir un système d'identification des agents du Groupe de risque 3 qui intéressera les FC dans le cadre du projet de centre de référence pour la défense contre les agents infectieux (CRDAI).

Bien que les cellules végétatives de *Bacillus anthracis* se soient révélées sensibles aux dilutions du Savlon et à la dilution 1:100 d'eau de Javel, ses spores étaient très résistantes. Toutefois, les spores de *Bacillus anthracis* sont détruites en moins d'une minute après un contact avec la dilution 1:10 d'eau de Javel. Les profils métaboliques Biolog™ ont été déterminés pour les 21 souches de *Bacillus anthracis* conservées au CRDS. Ces profils constitueront un élément important du système d'identification du CRDAI prévu. Les profils de sensibilité aux antibiotiques confirment que toutes les souches de l'agent du charbon bactéridien conservées au CRDS sont sensibles à divers antibiotiques, notamment la ciprofloxacine qui est le seul antibiotique approuvé par la FDA aux É.-U. pour le traitement du charbon pulmonaire.

Kournikakis, B.; Bateman, C.; Cherwonogrodzky, J.W. 2000. Characterization of 21 strains of *Bacillus anthracis*. TM-2000-157 Defence Research Establishment Suffield.

Table of contents

Abstract i

Résumé i

Executive summary iii

Sommaire iv

Table of contents v

List of figures vii

List of tables vii

Introduction 1

Materials and Methods 2

- Culture strains 2
- Disinfectant Sensitivity 2
- Antibiotic Sensitivity 2
- Biolog™ Metabolic Identification Profiles 3

Results and Discussion 4

- Culture of strains 4
- Disinfectant Sensitivities 6
- Biolog™ Metabolic Identification Profiles 6
- Antibiotic Sensitivities 9

Conclusion 10

References 11

Annex A - Biolog™ metabolic identification profiles of all *Bacillus anthracis* strains tested. 13

- *Bacillus anthracis*, Sterne strain (Thraxol) 13
- *Bacillus anthracis*, strain 93 - 189C 13
- *Bacillus anthracis*, strain 93 – 212C 14

Bacillus anthracis, strain 94 – 188C 14
Bacillus anthracis, strain 96 - 04 15
Bacillus anthracis, strain 96 - 09 15
Bacillus anthracis, strain 96 - 10 16
Bacillus anthracis, strain 96 - 19 16
Bacillus anthracis, strain 4229 17
Bacillus anthracis, strain RP42 (vaccine) 17
Bacillus anthracis, strain ACB 18
Bacillus anthracis, Ames strain 18
Bacillus anthracis, Buffalo strain 19
Bacillus anthracis, strain I7T5 19
Bacillus anthracis, strain NH 20
Bacillus anthracis, strain SK31 20
Bacillus anthracis, strain SK 61 21
Bacillus anthracis, strain SK 162 21
Bacillus anthracis, strain VH 22
Bacillus anthracis, Vollum strain 22
Bacillus anthracis, Vollum 1B strain 23

List of figures

Figure 1. *Bacillus anthracis* colonies on blood agar....4

Figure 2. *Bacillus anthracis* cells....5

List of tables

Table 1: *Bacillus anthracis* strains used in this study....5

Table 2: The effect of common disinfectants on *Bacillus anthracis* Sterne strain....6

Table 3: Substrate locations on Biolog GP-2 Plate....7

Table 4: *Bacillus anthracis* – Summary Metabolic Profile....8

Table 5. Antibiotic sensitivities for *Bacillus anthracis* strains....9

Introduction

Bacillus anthracis, the causative agent of anthrax, has long been known as the "classic" Biological Warfare (BW) agent. Its ease of growth, survival characteristics of its spore, its lethality in pneumonic form, and its past weaponization by several nations have all contributed to its reputation [1]. In light of this, it was certainly no surprise to learn that Iraq had weaponized this agent and caused great concern about its potential for use during the Gulf War Crisis in 1990.

As a disease, anthrax has received a great deal of press coverage since 1990 and has become a popular choice for incidents of "bioterrorism", although most of these cases to date have proven to be hoaxes [2]. In one incident, however, an American in Las Vegas declared that he had a vial of *Bacillus anthracis*, and indeed he did much to the dismay of authorities. It was later learned that the anthrax in question was the vaccine strain and not a virulent strain as the individual had implied [3].

With its potential as both a military and terrorist threat, *Bacillus anthracis* is an integral part of the research program at DRES. It is used as a model system in the study of medical countermeasures, identification and decontamination studies. As part of our safety protocols we have undertaken to characterize the antibiotic and disinfectant sensitivities of *Bacillus anthracis*, as well as the other Risk Group 3 bacterial agents at DRES. This ensures that this information will be quickly available in the event of accidental exposure at DRES, and available to the Canadian Forces (CF) and other government agencies in the event of military or terrorist use. We are also in the process of establishing an identification capability for Risk Group 3 agents of interest to the CF as part of the proposed Defence Reference Center for Infectious Agents (DRCIA).

In this report, we present our findings on the culture, disinfectant and antibiotic sensitivities, and Biolog™ metabolic profiles of 21 strains of *Bacillus anthracis*.

Materials and Methods

Culture strains

Strains of *Bacillus anthracis* were acquired from 3 institutions, the Animal Disease Research Institute – Lethbridge, Alberta (ADRI-Lethbridge), the United States Research Institute for Infectious Diseases (USAMRIID, Frederick, MD, USA) and the Pasteur Institute (Paris, France). We are grateful for their contributions. Table 1 shows an inventory of our *Bacillus. anthracis* stocks.

Stocks were stored at –70° C, in vials with 25 Protect Beads™ (TSC Ltd., UK) and/or 0.9 mL trypticase soy broth with 0.1 mL glycerol. Stocks were subcultured onto commercial plates of trypticase soy agar supplemented with 5% sheep red blood cells (blood agar plates or BAP) (PML Microbiologicals, Wilsonville, OR, USA) and grown at 37° C, 5% CO2, 90% humidity. For the Biolog™ assay, strains were subcultured onto chocolate agar (PML Microbiologicals) as specified in the Biolog™ manual.

Disinfectant Sensitivity

Bacillus anthracis Sterne strain was used as a representative strain to test survival of vegetative cells (i.e. a culture grown overnight on BAP) or spores (i.e. a culture that was left in the incubator for 1 month and checked by gram staining and microscopic examination to confirm that most cells had gone into the spore state). Bacteria were scraped from agar plates and suspended in 100 ml of sterile 1% saline to prepare a bacterial suspension with an OD of 0.17 at 620 nm (which corresponds to a MacFarlane tube standard of 1.0). Nine ml of the suspension was added to each of 5 tubes containing 1 ml of either sterile saline, 1:10 diluted Savlon (trade name for a medical disinfectant containing Chlorhexidine Gluconate which is a bisbiguanide antiseptic and disinfectant and Cetrimide which is a quaternary ammonium antiseptic), 1:100 diluted Savlon, 1:10 diluted household bleach, or 1:100 diluted houshold bleach. At noted times, 1 ml samples were removed, serially diluted and plated in duplicate on BAP. Plates were then incubated overnight at 37° C. and the colonies counted on each plate to determine the number of colony forming units (CFU) for each sample.

Antibiotic Sensitivity

Procedures used were as noted in the National Committee of Clinical Laboratory Studies (NCCLS) guidelines in the Clinical Microbiology Procedures Handbook [4].

Biolog™ Metabolic Identification Profiles

Procedures used were as noted in the Biolog™ Manual or as recommended by the Biolog™ Inc. Cultures were subcultured 3 times on chocolate agar, the fourth subculture was done as late as possible on one day and used as early as possible on the next day to ensure that cells were in the early phase of growth. Cells at the edge of growth, or if required from lone colonies, were harvested with a sterile toothpick and resuspended to the optical absorbance recommended. Unlike other *Bacillus* species, *B. anthracis* did tend to floculate and settle. For this reason the suspension was mixed vigorously and dispensed immediately into the GP-2 96-well metabolic plates. Colour changes were read after 4-6 hours incubation and again after 16 hours incubation. Results shown are from single assays of each strain tested.

Results and Discussion

Culture of strains

Table 1 is a list of *Bacillus anthracis* strains currently held at DRES and used in this study. Upon plating on various media, this bacterium is very hardy, growing readily and rapidly on trypticase soy agar, peptone medium, nutrient agar, etc. Many pathogenic bacteria are fastidious and grow poorly on nutrient agar. In contrast, *Bacillus anthracis* grows well, has a characteristic unpigmented to dark gray rough appearance and is often readily distinguishable from common contaminants. Aside from Vollum and Vollum 1B cells that appeared more "crusty", the colonial morphology of most strains was indistinguishable, having a spreading "Greenland" appearance on the plates (Fig. 1).

Figure 1. *Bacillus anthracis* colonies on blood agar

Fig. 2 shows a gram stain of a 2 day old culture of *Bacillus anthracis*. The bacterial spores (examples circled) are beginning to appear in these cells. When a gram stain is prepared from a 2 week old culture (not shown) only the glassy bead-like spores and cell debris are evident on microscopic examination. It should also be noted that despite going through the gram stain process, it was still possible to isolate viable cells from the slide preparation, a clear indication of the ability of the bacterial spore to survive adverse conditions.

Figure 2. *Bacillus anthracis* cells

Table 1: *Bacillus anthracis* strains used in this study		
***Bacillus anthracis* strain**	***Source***	***History of isolate***
Thraxol (Sterne vaccine)	ADRI-Lethbridge	Vaccine
93-189C	ADRI-Lethbridge	Bison, Hay River
93-212C	ADRI-Lethbridge	Raven, Mackenzie Bison Sanctuary , NWT
94-188C	ADRI-Lethbridge	Bovine, Avonlea, Saskatchewan
96-04	ADRI-Lethbridge	Bovine, Sibbald, Alberta
96-09	ADRI-Lethbridge	Bovine, High Level ,Alberta
96-10	ADRI-Lethbridge	Bovine, Iroquois, Ontario
96-19	ADRI-Lethbridge	Bovine, Westlock, Ontario
4229	ADRI-Lethbridge	ATCC, Capsule +, toxin -
RP42 (Vaccine)	Pasteur Institute	PA+, EF-, LF-
ACB	USAMRIID	Human, Ohio, 1952
Ames	USAMRIID	Cow, Iowa, 1980
Buffalo	USAMRIID	Buffalo, Iowa, 1979
17T5	USAMRIID	Kudu, S. Africa, 1957
NH	USAMRIID	Human, New Hampshire, 1957
SK31	USAMRIID	Wildebeest, S. Africa, 1974
SK61	USAMRIID	Human, California, 1976
SK162	USAMRIID	Human, Florida, 1976
VH	USAMRIID	Human, S. Africa, 1952
Vollum	USAMRIID	Cow, 1944
Vollum 1B	USAMRIID	From Vollum

Disinfectant Sensitivities

Table 2 summarizes the effect of some common disinfectants on *Bacillus anthracis* Sterne strain (vegetative cells and spores). It can be readily seen that unlike the vegetative cells which are sensitive to the disinfectants tested, the spores of *Bacillus anthracis* are resistant to everything but 1:10 diluted bleach.

Table 2: The effect of common disinfectants on *Bacillus anthracis* Sterne strain

	Exposure Time	Disinfectant				
		Control	1.10 diluted Savlon	1.100 diluted Savlon	1:10 diluted bleach	1:100 diluted bleach
Vegetative cells	< 1 min.	3.5×10^8	$<10^3$	$<10^3$	$<10^3$	$<10^3$
	1 hr.	3.7×10^8	$<10^3$	$<10^3$	$<10^3$	$<10^3$
	2 hrs.	3.7×10^8	$<10^3$	$<10^3$	$<10^3$	$<10^3$
Spores	< 1min	9.4×10^8	2.9×10^8	2.7×10^8	$<10^3$	1.0×10^7
	1hr	9.7×10^8	3.4×10^8	3.7×10^8	$<10^3$	0.5×10^7
	2 hrs	7.3×10^8	1.8×10^8	1.8×10^8	$<10^3$	2.0×10^6

Results shown are colony forming units which grew on BAP following plating after noted incubation times in the disinfectants

Biolog™ Metabolic Identification Profiles

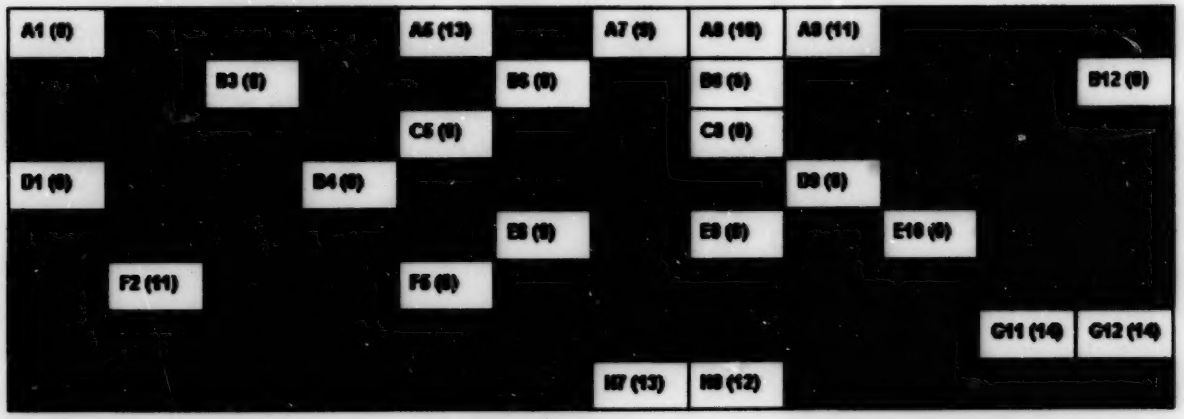
Table 3 shows the metabolites in each of the different wells of the Biolog™ GP-2 microtitre plate used in this study. Appendix A gives the metabolic profiles for all anthrax strains tested. Table 4 gives a summary of all the profiles from the tested strains, showing which wells were positive for 1-7, 8-15, and 16-21 of strains tested, as well as those wells which were negative for all strains tested. These metabolic "fingerprints" will be an important part of our DRCIA identification capability for this agent together with other diagnostic criteria normally used for anthrax. Clear similarities were noted to the Biolog™ data base (not shown) for *Bacillus cereus* and *Bacillus thuingensis*. This metabolic profile similarity is not surprising considering the close genetic relationship between these organisms [5].

It is also noteworthy that there is considerable variation in the metabolic profiles of the different anthrax strains. In discussions with Biolog (who incorporated our data, along with that of others into its overall database) we learned that it was necessary to create 4 groupings of anthrax strains based on the diversity of the metabolic "fingerprints" seen.

It is curious to note that the metabolic profile of the deletion mutant RP42 [6] (Annex A), which lacks the ability to make lethal factor, edema factor and capsules, is more metabolically active (using all substrates in 16-24 hr incubation period) than its parent Sterne strain (which uses only 14 substrates).

Table 3: Substrate locations on Biolog GP-2 Plate			
Well	***Substrate***	***Well***	***Substrate***
A1	Water	E1	D-tagatose
A2	α-cyclo-dextrin	E2	D-trehalose
A3	β-cyclo-dextrin	E3	turanose
A4	dextrin	E4	xylitol
A5	glycogen	E5	D-xylose
A6	inulin	E6	Acetic acid
A7	mannin	E7	α-hydroxy-butyric acid
A8	Tween 40	E8	β-hydroxy-butyric acid
A9	Tween 80	E9	γ-hydroxy-butyric acid
A10	N-acetyl-D-glucos-amine	E10	p-hydroxy-phenyl acetic acid
A11	N-acetyl-D-mannos-amine	E11	α-keto glutaric acid
A12	amygdalin	E12	α-keto valeric acid
B1	L-arabinose	F1	Lactamide
B2	D-arabitol	F2	lactic acid methyl ester
B3	arbutin	F3	L-lactic acid
B4	cellobiose	F4	D-malic acid
B5	D-fructose	F5	L-malic acid
B6	L-fucose	F6	methyl pyruvate
B7	D-galactose	F7	mono-methyl succinate
B8	D-galacturonic acid	F8	propionic acid
B9	gentiobiose	F9	pyruvic acid
B10	Gluconic acid	F10	succinamic acid
B11	α-D-glucose	F11	succinic acid
B12	m-inositol	F12	N-acetyl L-glutamic acid
C1	α-D-lactose	G1	alaninamide
C2	lactulose	G2	D-alanine
C3	Maltose	G3	L-alanine
C4	Maltotriose	G4	L-alanyl-glycine
C5	D-mannitol	G5	L-asparagine
C6	D-mannose	G6	L-glutamic acid
C7	D-melezitose	G7	Glycyl-L-glutamic acid
C8	D-melibiose	G8	L-pyro-glutamic acid
C9	α-methyl D-galactoside	G9	L-serine
C10	β-methyl D-galactoside	G10	Putresine
C11	3-methyl glucose	G11	2,3-butanediol
C12	α-methyl D-glucoside	G12	glycerol
D1	β-methyl D-glucoside	H1	Adenosine
D2	α-methyl D-mannoside	H2	2'-deoxyadenosine
D3	Palatinose	H3	Inosine
D4	D-psicose	H4	Thymidine
D5	D-raffinose	H5	Uridine
D6	L-rhamnose	H6	Adenosine-5'-monophosphate
D7	D-ribose	H7	Thymidine-5'-monophosphate
D8	Salicin	H8	Uridine-5'monophosphate
D9	Sedoheptulosan	H9	Fructose-6-phosphate
D10	D-sorbitol	H10	Glucose-1-phosphate
D11	Stachyose	H11	Glucose-6-phosphate
D12	Sucrose	H12	D-L-α-glycerol phosphate

Table 4: *Bacillus anthracis* – Summary Metabolic Profile



Wells shown in red were positve in 15 to 21 of the 21 anthrax strains tested Wells shown in yellow were positive for 8 to 14 of the 21 strains tested. Wells shown in green were only positive for 1 to 7 of the 21 anthrax strains tested Wells in white were negative for all 21 strains tested. Numbers in parentheses following the well designator indicate the actual number of the 21 strains which tested positive in that well.

Antibiotic Sensitivities

Table 5 shows a summation chart of the antibiotic sensitivies for all 21 strains of *Bacillus anthracis* tested. Most strains are sensitive to several antibiotics. These profiles, possibly in unison with the antibiotic profiles of other BW agents, should be considered when antibiotics are prescribed as prophylactic or therapeutic measures against *Bacillus anthracis*. An FDA advisory committee in the US recommended [7] approval of ciprofloxacin for treatment of inhalation anthrax. All DRES strains were found to be sensitive to ciprofloxacin.

Table 5. Antibiotic sensitivities for *Bacillus anthracis* strains

Antibiotic Family	Antibiotic	Code and amt (Mcg)	Sensitivity (S) or Resistance (R)
Aminoglycoside	Amikacin	AN 30	S (21/21) 100%
	Gentamycin	GM 10	S (21/21) 100%
	Kanomycin	K 30	S (21/21) 100%
	Neomycin	N 30	S (20/21) 95%, R (1/21) 5%
	Streptomycin	S 10	S (21/21) 100%
	Tobramycin	TM 10	S (21/21) 100%
Beta Lactams & Penicillins	Ampicillin	AM 10	S (21/21) 100%
	Carbenicillin	CB 100	S (21/21) 100%
	Pipercillin	PIP 100	S (21/21) 100%
	Ticaracillin	TIC 75	S (21/21) 100%
	Oxacillin	OX 1	S (21/21) 100%
Cephalosporins & Cephans	Cefotaxime	CTX 30	S (3/21) 15%, I (7/21) 33%, R (11/21) 52%
	Ceftazidime	CAZ 30	R (21/21) 100%
	Cethalopin	CR 30	S (21/21) 100%
Lincosamide	Clindamycin	CC 2	S (20/21) 95%, R (1/21) 5%
Macrolides	Erythromycin	E 15	S (20/21) 95%, R (1/21) 5%
Others	Chloramphenicol	C 30	S (21/21) 100%
	Colistin	CL 10	R (21/21) 100%
	Nitrofurantoin	FD 300	S (20/21) 95%, I (1/21) 5%
	Novobiocin	NB 30	S (21/21) 100%
	Rifampin	RA 5	S (19/21) 90%, I (1/21) 5%, R (1/21) 5%
	Vancomycin	VA 30	S (21/21) 100%
Peptide	Bacitracin	B 10 UNITS	S (1/21) 5%, I (18/21) 86%, R (2/21) 9%
Polypeptides	PolymixonB	PB 300	S (5/21) 24%, I (10/21) 48%, R (6/21) 28%
Quinolones	Ciprofloxacin	CIP 5	S (21/21) 100%
	Nalidixic acid	NA 30	S (21/21) 100%
	Norfloxacin	NOR 10	S (21/21) 100%
Sulfonamide	Sulfisoxazole	G 300	S (21/21) 100%
Sulfonamides	Trimethoprim	SxT 25	S (21/21) 100%
Tetracyclines	Doxycycline	DO 30	S (21/21) 100%
	Tetracycline	TE 30	S (21/21) 100%

Conclusion

Although vegetative cells of *B. anthracis* were sensitive to diluted Savlon or diluted bleach, its spores are very resistant to Savlon or to 1:100 household bleach. Spores, however, are destroyed in less than a minute after coming into contact with 1:10 household bleach. Biolog™ metabolic profiles were determined for all 21 anthrax strains held at DRES. These profiles will serve as a key part of planned DRCIA identification capabilities. Antibiotic sensitivity profiles confirm that all anthrax strains held at DRES are sensitive to a variety of antibiotics, including Ciprofloxacin which is the only antibiotic approved by the FDA in the US for treatment of inhalational anthrax.

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Annex A - Biolog™ metabolic identification profiles of all *Bacillus anthracis* strains tested

Bacillus anthracis, Sterne strain (Thraxol)

A1	A2	A3	[redacted]	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	[redacted]	B12
C1	C2	[redacted]	C4	C5	[redacted]	C7	C8	C9	C10	[redacted]	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	[redacted]		E4	E5	E6	E7	E8	E9	E10	E11	[redacted]
F1	F2	F3	F4	F5	[redacted]	F7	F8	[redacted]	F10	F11	F12
G1	G2	G3	G4	[redacted]		G7	G8	G9	G10	G11	G12
[redacted]	H2	[redacted]	H4	H5	H6	H7	H8	H9	H10	H11	H12

Bacillus anthracis, strain 93 - 189C

A1	A2	[redacted]	[redacted]	[redacted]	A6	[redacted]	[redacted]	[redacted]	A10	A11	A12
B1	B2	B3	B4	[redacted]	B6	B7	B8	[redacted]	B10	[redacted]	B12
[redacted]	[redacted]	[redacted]	[redacted]	C5	[redacted]	C7	C8	[redacted]	C10	[redacted]	C12
D1	[redacted]	[redacted]	[redacted]	D5	[redacted]	[redacted]	D8	D9	D10	D11	[redacted]
E1	[redacted]	E3	E4	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	E10	E11	[redacted]
[redacted]	[redacted]	[redacted]	F4	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
G1	G2	[redacted]	[redacted]	[redacted]	[redacted]	G7	G8	[redacted]	G10	[redacted]	[redacted]
[redacted]	[redacted]	H3	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	H12

***Bacillus anthracis*, strain 93 – 212C**

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3		D5	D6		D8	D9	D10	D11	D12
E1			E4	E5				E9	E10	E11	
			F4			F7	F8				
	G2					G7	G8				
								H9	H10	H11	H12

***Bacillus anthracis*, strain 94 – 188C**

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

***Bacillus anthracis*, strain 96 - 04**

A1	A2	A3		A5	A6	A7	A8	A9		A11	A12
B1	B2	B3	B4		B6	B7	B8		B10	B11	B12
C1	C2			C5		C7	C8	C9	C10		C12
D1	D2			D5	D6	D7	D8	D9	D10	D11	
E1		E4	E5	E6		E8	E9	E10	E11		
	F2		F4	F5		F7	F8		F10	F11	F12
	G2					G7	G8		G10	G11	G12
								H9	H10	H11	H12

***Bacillus anthracis*, strain 96 - 09**

A1	A2	A3		A5	A6	A7	A8	A9		A11	A12
B1	B2	B3	B4		B6	B7	B8		B10	B11	B12
C1	C2			C5		C7	C8	C9	C10		C12
D1	D2		D4	D5	D6	D7	D8	D9	D10	D11	
E1			E4	E5	E6			E9	E10	E11	
F1	F2		F4	F5		F7	F8		F10	F11	F12
	G2					G7	G8		G10	G11	
								H9	H10	H11	H12

Bacillus anthracis, strain 96 - 10

A1	A2 +		A4 +	A5 +	A6	A7 +			A10 +	A11	A12 +
	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
		C3	C4	C5	C6	C7	C8	C9	C10	C11 +	C12
D1	D2	D3	D4		D6		D8	D9	D10	D11	D12
E1	E2	E3	E4		E6	E7	E8	E9	E10	E11	
F1	F2	F3	F4	F5		F7	F8		F10	F11	F12
	G2	G3	G4	G5	G6	G7	G8		G10		
				H5	H6	H7	H8		H10	H11	H12

Bacillus anthracis, strain 96 - 19

A1	A2	A3			A6	A7	A8	A9		A11	A12
B1	B2	B3	B4		B6	B7	B8		B10	B11 +	B12
C1	C2		C4 +	C5	C6 +	C7	C8	C9	C10	C11 +	C12
D1	D2		D4	D5	D6	D7 +	D8	D9	D10	D11	D12 +
E1	E2 +		E4	E5	E6	E7 +	E8	E9	E10	E11	E12 +
F1	F2 +		F4	F5 +	F6 +	F7 +	F8	F9 +	F10	F11	F12
G1 +	G2			G5 +	G6 +	G7	G8		G10	G11 +	G12 +
H1 +	H2 +					H7 +		H9	H10	H11	H12

Bacillus anthracis, strain 4229

A1	A2	A3		A5	A6	A7	A8	A9		A11	A12
B1	B2	B3	B4		B6	B7	B8	B9	B10		B12
C1	C2			C5		C7	C8	C9	C10		C12
D1	D2		D4	D5	D6	D7	D8	D9	D10	D11	D12
E1			E4	E5	E6	E7	E8	E9	E10	E11	
F1	F2	F3	F4	F5		F7	F8		F10	F11	F12
G1	G2					G7	G8	G9	G10	G11	G12
					H6	H7	H8		H10		H12

Bacillus anthracis, strain RP42 (vaccine)

A1	A2			A5	A6	A7	A8			A11	A12
B1	B2	B3			B6	B7	B8		B10		B12
C1	C2			C5		C7	C8		C10		C12
D1			D4	D5	D6			D9	D10	D11	
E1			E4	E5	E6		E8	E9	E10	E11	
F1			F4	F5		F7	F8		F10	F11	F12
G1	G2				G6	G7	G8		G10		
								H9	H10	H11	

Note: Results are recorded from a 4 – 6 hour incubation time plate. The results from this plate were more discriminating than the 16 – 24 hour incubation plate, which showed ~ 97% positive well reactions.

***Bacillus anthracis*, strain ACB**

A1	A2 +				A6 +	A7	A8 +		A10 +	A11	A12
B1	B2 +	B3		B5 +	B6	B7 +	B8		B10 +	B11 +	B12
C1				C5	C6 +	C7	C8	C9		C11 +	
D1	D2	D3	D4	D5	D6	D7	D8	D9		D11	
E1						E7	E8 +	E9	E10	E11	E12
	F2			F5			F8				
	G2				G6	G7	G8		G10		
				H5	H6	H7	H8	H9	H10	H11	H12

***Bacillus anthracis*, Ames strain**

A1	A2				A6 +	A7	A8 +			A11	A12
B1	B2	B3		B5	B6	B7	B8	B9 +	B10	B11	B12
C1	C2			C5	C6 +	C7	C8	C9	C10	C11 +	C12
D1	D2		D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2 +		E4	E5	E6	E7	E8	E9	E10	E11	E12
	F2 +	F3	F4			F7	F8		F10	F11	F12
	G2 +		G4	G5	G6 +	G7	G8 +	G9	G10	G11	G12
	H2 +	H3		H5 +	H6	H7	H8	H9	H10	H11	H12

***Bacillus anthracis*, Buffalo strain**

A1	A2				A6					A11	
B1	B2	B3	B4		B6		B8		B10		B12
C1	C2			C5		C7	C8	C9	C10		C12
D1	D2		D4	D5				D9	D10	D11	D12
E1			E4	E5	E6		E8	E9	E10	E11	
F1			F4	F5	F6	F7	F8		F10	F11	
	G2	G3		G5	G6	G7	G8	G9	G10	G11	G12
				H5		H7		H9	H10	H11	H12

***Bacillus anthracis*, strain I7T5**

A1	A2				A6						
B1	B2	B3	B4		B6	B7	B8				B12
C1				C5		C7	C8		C10		C12
D1	D2		D4	D5	D6		D8	D9	D10		
E1			E4	E5			E8	E9	E10		
F1	F2+	F3+	F4	F5+	F6+	F7+	F8	F9+	F10+	F11+	F12+
G1+	G2	G3+	G4	G5+	G6+	G7+	G8+	G9+	G10	G11+	G12+
H1+	H2+	H3+	H4+	H5+	H6+	H7+	H8+	H9	H10	H11	H12+

***Bacillus anthracis*, strain NH**

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

***Bacillus anthracis*, strain SK31**

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

***Bacillus anthracis*, strain SK 61**

A1	A2					A7					
	B2	B3	B4	B5	B6	B7	B8				B12
	C2			C5		C7	C8	C9			C12
D1		D3	D4	D5	D6		D8	D9	D10	D11	
			E4					E9	E10		
F1			F4	F5	F6	F7	F8		F10		F12
				G5		G7	G8		G10		
				H5			H8		H10	H11	H12

***Bacillus anthracis*, strain SK 162**

A1				A5	A6					A11	A12
B1	B2	B3	B4		B6	B7	B8		B10		B12
C1	C2			C5		C7	C8	C9			C12
D1	D2						D8	D9	D10	D11	D12
			E4	E5				E9	E10	E11	
F1			F4			F7	F8		F10	F11	
	G2					G7	G8		G10		
						H7			H10		

***Bacillus anthracis*, strain VH**

A1						A7				A11	A12
B1	B2	B3			B6	B7	B8	B9	B10		B12
C1				C5	C6	C7	C8	C9	C10	C11	C12
D1		D3		D5	D6	D7	D8	D9	D10	D11	D12
E1						E7		E9	E10	E11	
			F4		F6	F7	F8		F10	F11	F12
	G2	G3	G4		G6	G7	G8		G10	G11	G12
				H5			H8	H9	H10	H11	H12

***Bacillus anthracis*, Vollum strain**

A1	A2	A3		A5	A6	A7	A8	A9		A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10		B12
C1	C2		C4	C5		C7	C8	C9	C10	C11	C12
D1	D2		D4	D5	D6	D7	D8	D9	D10	D11	
E1			E4	E5	E6	E7	E8	E9	E10	E11	
F1	F2	F3	F4	F5		F7	F8		F10	F11	F12
	G2					G7	G8		G10		
								H9	H10	H11	H12

***Bacillus anthracis*, Vollum 1B strain**

A1					A6		A8	A9		
B1	B2	B3			B6	B7	B8			B12
C1	C2			C5		C7	C8	C9	C10	C12
D1	D2				D6		D8	D9	D10	D11
			E4	E5			E8	E9	E10	
F1			F4				F8		F10	F12
						G7	G8		G10	
								H9	H10	H11

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14 ABSTRACT

(U) Twenty-one strains of Bacillus anthracis currently held in the culture collection at DRES were characterized by colonial morphology, antibiotic sensitivity and Biolog™ metabolic identification profiles. Aside from Bacillus anthracis strain Vollum which appeared more "crusty" on blood plates, the other strains were similar, giving a characteristic "Greenland" (rough edge, off-white to tan) appearance Most strains were resistant to ceftazidime, colistin, bacitracin, polymyxin B and sensitive to the other antibiotics tested, such as ciprofloxacin. Biolog™ metabolic identification profiles were prepared for each of the 21 strains of Bacillus anthracis as part of the identification database being developed for the "Defence Reference Center for Infectious Agents (DRCIA) (a center of expertise for endemic and exotic diseases of concern to the Canadian Forces) at DRES A representative strain (Sterne) of Bacillus anthracis was used to test the disinfectant sensitivity of both Bacillus anthracis vegetative cells and spores. Bacillus anthracis vegetative cells were sensitive to 1:100 and 1:10 diluted Savlon™ as well as 1 100 and 1 10 diluted household bleach while the spores were only sensitive to 1:10 diluted household bleach

15. KEYWORDS, DESCRIPTORS or IDENTIFIERS

(U) Anthrax; Bacillus anthracis, identification; Biolog; antibiotic sensitivity